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Arthrobotrys latispora, a new nematode-trapping fungus from southwest China

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Abstract — Using morphology and molecular phylogenetic analyses, we report a new nematophagous hyphomycete species, *Arthrobotrys latispora,* which produces erect, branched or unbranched conidiophores with conspicuous nodes at the tip; the conidia are nonseptate (41%) or uniseptate (59%) and broadly ovoid to oval. Three-dimensional adhesive networks formed in the presence of nematodes.

Key words — teleomorph–anamorph connection, *Orbilia*

Introduction

Nematode-trapping fungi have been the subject of decades-long research, including their ecology, distribution and systematics, due in part to their potential as biological control agents to manage plant and animal nematode diseases (Liu et al. 2002, Liu & Zhang 2003, Dong et al. 2004). These fungi are able to trap and consume nematodes with modified hyphae that include stalked and sessile adhesive knobs, adhesive nets, and constricting or non-constricting rings. Species forming adhesive nets are assigned to the anamorphic genus *Arthrobotrys* Corda, which currently comprises about 50 species (Scholler et al. 1999).

Pfister (1994) was the first to connect an *Arthrobotrys* species to the ascomycetous genus *Orbilia* on the basis of cultural studies. Up to now, six species of *Arthrobotrys* have been linked to related *Orbilia* teleomorphs. *Orbilia auricolor* (A. Bloxam) Sacc., seemingly a species complex, is known to have four anamorphs: *A. oligospora* Fresen., *A. cladodes* Drechsler (Pfister & Liftik 1995), *A. yunnanensis* M.H. Mo & K.Q. Zhang (Mo et al. 2005), and *A. psychrophila* (Drechsler) M. Scholler et al. (Rubner 1996). Of the remaining two species, *O.*

fimicola Jeng & J.C. Krug pairs with *A. superba* Corda (Pfister 1994) and an undescribed *Orbilia* sp. with *A. nonseptata* Z.F. Yu et al. (Li et al. 2009).

A survey of *Orbilia* species and their anamorphs revealed one *Arthrobotrys* species that had not yet been characterized. As neither morphological comparison nor phylogenetic analysis of the internal transcribed spacer (ITS) regions of rRNA genes could assign this isolate to a previously named taxon, it is reported here as a new species, *Arthrobotrys latispora*.

Materials & methods

Collection, isolation, and characterization of teleomorph fungi and their anamorphs

Fresh specimens of an *Orbilia* species were collected on decaying bark of *Castanopsis orthacantha* Franch. (*Fagaceae*), located in the northwest part of the YongPing County (25°7′18″–25°13′44″N 99°27′26″–99°36′07″E), Yunnan Province, China where the dominant plant species are *Castanopsis orthacantha*, *Lithocarpus variolosus* Chun, *Cyclobalanopsis glauca* Oerst., and *Cinnamomum glanduliferum* (Wall.) Meisn. Specimens are preserved in the herbarium of the Biology and Chemistry College of Dali University, China.

To cultivate the fungus, four apothecia were fixed to the lid of a Petri dish with hymenia positioned downward in order to collect discharged ascospores on the surface of CMA media (20 g corn meal, 18 g agar, 40 mg streptomycin, and 30 mg ampicillin in 1000 ml distilled water). These Petri dishes were placed at room temperature for 4–6 days until the ascospores germinated. Agar blocks with deposited ascospores were transferred to another CMA plate to avoid contamination and incubated at 25°C. After 10–15 days, the cultures were examined with an Olympus BX51 microscope with differential interference contrast. Trapping organs were induced by adding ~100 nematodes (*Panagrellus redivivus* Goodey) to a square slot (1 × 1 cm) created by removing the agar at the colony edge.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from the mycelia grown on cellophane membrane on PDA according to Jeewon et al. (2002). Primer pairs ITS5 and ITS4 (White et al. 1990) were used to amplify the complete ITS + 5.8S region. PCR amplification parameters were: 1 minute initial denaturation at 95°C, followed by 30 cycles of 1 minute denaturation at 94°C, 1 minute primer annealing at 50°C, 1.5 minutes extension at 72°C, and a final extension period of 10 minutes at 72°C. The purified PCR products were directly sequenced in forward and reverse directions using the same PCR primers (BGI Co., Ltd., China).

Phylogenetic analysis

Complete ITS1+5.8S+ITS2 rDNA sequences were aligned with 20 sequences (obtained from GenBank, NCBI [http://www.ncbi.nlm.nih.gov/]) by MUSCLE (MUltiple Sequence Comparison by Log-Expectation) at the EBI (European Bioinformatics Institute) web server [http://www.ebi.ac.uk/Tools/muscle/index.html]. ITS4 and ITS5 sequences were excluded from the analysis of all taxa. The DNA matrix of 21 taxa and 496 nucleotides was adjusted manually to improve the alignment. Phylogenetic analysis

was conducted with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Alignment gaps were treated as missing data; the sequence file set as interleaved; and GTR model was used for the substitution model. Markov chains were run for 1,000,000 generations, and trees were sampled every 10th generation resulting in 100,000 trees. About 0.003 average standard deviation of split frequency was achieved. The first 25,000 burn-in phase trees were discarded while the remaining 75,000 trees were used for calculating posterior probabilities in the consensus tree. The consensus tree was analyzed by the program FigTree v1.3.1. (Rambaut & Drummond 2010). The GenBank accession numbers of all the analyzed sequences were indicated after names of the species on the phylogenetic tree.

Results

Phylogenetic analysis

The Bayesian tree generated from the ITS1+5.8S+ITS2 rDNA sequences of *Arthrobotrys latispora* and its relative species (shown in Plate 1) indicates that *Arthrobotrys* and *Drechslerella* species group into two different clades, with *Dactylellina* species basal to both. The Bayesian posterior probabilities from the ITS genotyping resulting in these three major groups correspond to the different trapping device types and are consistent with the results of Scholler et

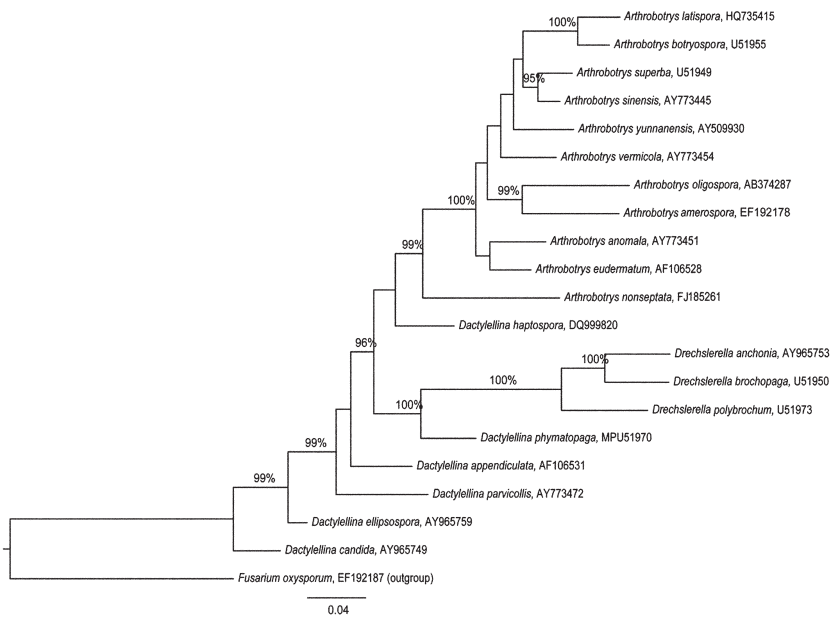

Plate 1. Bayesian tree derived from complete ITS1, ITS2 and 5.8S rDNA sequences of nematode-trapping fungi with *Fusarium oxysporum* set as outgroup. Bayesian posterior probabilities over 95% are shown on the branches, and scale bar at the bottom shows the expected changes per site.

Table 1. Morphology of *Arthrobotrys* species with non-septate conidia.

Species	Conidia			Conidiogen. denticle	Conidiophore (height µm)	Predacious organs
	Shape	Size (µm)	Septa (#)			
A. amerospora	obovoid	15–31 × 10–20	0	short	75–250	adhesive networks
A. anomala	cylindric to long ellipsoid	13–22 × 3–7	0 (–1)	short	20–80	adhesive branches & networks
A. arthro-botryoides sensu Drechsler	broadly cylindric-ellipsoid	17–30 × 10–16	(0–) 1	short	300–450	adhesive networks
A. botryospora	ellipsoid	12–20 × 11–15	0 (–1)	short	250–450	adhesive hyphae & networks
A. latispora	broadly ovoid-oval	14.8–21.5 × 10.1–16.3	0–1	short	60–250	adhesive networks
A. nonseptata	elongate ellipsoid	11–16.8 × 5–6.6	0	short	40–120	adhesive networks
A. yunnanensis	elongate, ellipsoid-cylindric, or clavate	17.5–32.5 × 2.75–7.5	0(–1)	long	60–200	adhesive networks

al. (1999). The trapping devices of *Arthrobotrys*, *Dactylellina,* and *Drechslerella* species form, respectively, adhesive networks, adhesive knobs, and constricting rings. *Arthrobotrys latispora* is situated within the adhesive networks clade and appears closely related to *A. botryospora* G.L. Barron. Morphological comparisons are shown in Table 1. Because our isolate differs from previously described *Arthobotrys* species, we propose it here as a new species.

Arthrobotrys latispora H.Y. Su & X.Y Yang, **sp. nov.** Plate 2

Coloniae in CMA effusae, ad 6 cm diam. post 8 dies 25°C. Mycelium sparsum, effusum, hyalinum, septatum, romosum, 2–4 µm laum. Conidiophora hyalina, simplicia, erecta, septata, non ramosa, plerumque 60–210 µm alta, basi 2–5 µm crassa, apice 1.8–4 µm crasso, efferenti 4–12 conidia sola de conidiogenis loci in perspicuis dendriculis in apicie aut prope apicem. Conidia hyalina, elongato ellipsoideo-cylindrica vel clavata, non- vel uniseptata, 14.8–21.5 × 10.1–16.3 (x = 18.99 × 13.5) µm. Reticula tenacia quae vermiculos nematodeos capiunt evolventibus. Chlamydosporae globosae vel ellipsoideae, catenulatae.

Holotype: Dried agar plate of a culture isolated from a collection of an undescribed *Orbilia* teleomorph [see below], (**holotype**, YMF 1.03168; ex-type culture, YMF 1.03168; **isotype** and ex-type culture, College of Agriculture and Biology, Dali University).

Etymology: '*latispora*' refers to the broadly ovoid conidia.

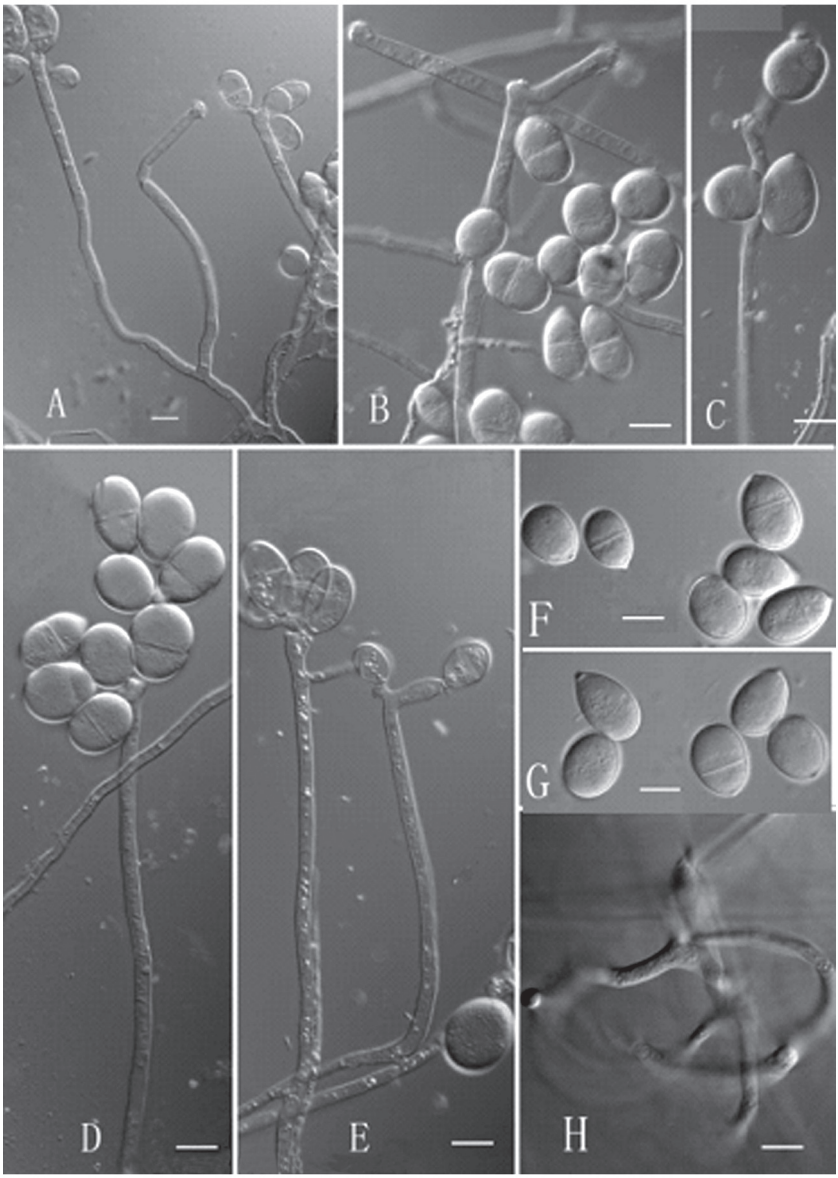

Plate 2. *Arthrobotrys latispora*:
A–E. Conidiophores with spore. F–G. Conidia. H. Adhesive nets. Bars: A–H =10 μm.

Assumed teleomorph: an unidentified *Orbilia* sp., YMFT 1.03168, collected on decaying bark of *Castanopsis orthacantha* (*Fagaceae*), PR CHINA, Yunnan Province, YongPing County, Jinguangsi Virgin Forest, alt. 2820 m, 20 september 2008, coll. X.J. Su, Z.L. Luo & H.Y. Su. (Duplicate material deposited at College of Agriculture and Biology, Dali University.)

Colonies grew rapidly on CMA medium, attaining 6 cm diam. in 8 days at 25°C. Mycelium spreading, vegetative hyphae hyaline, septate and branched, mostly 2–4 µm wide. Conidiophores colorless, erect, simple, septate frequently, 60–210 µm high, 2–5 µm wide at the base and 1.8–4 µm at the tip, producing 4–12 conidia singly from conidiogenous loci on conspicuous, partly superposed nodes at and near the apex. Conidia colorless, broadly ovoid-oval, broadly rounded at the tip, rounded truncate at the narrowed base, uniseptate or nonseptate at the center, 14.8–21.5 × 10.1–16.3 (x = 18.3 × 13.5) µm (living state). Approximately 41% of the conidia were nonseptate and 59% uniseptate. Chlamydospores spherical to ellipsoidal. Nematodes are trapped by three-dimensional adhesive networks.

Discussion

There are six known species of *Arthrobotrys* with nonseptate conidia, some of which form both uni- and nonseptate conidia. Conidia of *A. amerospora* (Schenck et al. 1977) and *A. nonseptata* (Li et al. 2009) are consistently nonseptate while those of *A. anomala* (Barron & Davidson 1972), *A. botryospora* (Barron 1979), and *A. yunnanensis* (Mo et al. 2005) are occasionally uniseptate. *A. arthrobotryoides* sensu Drechsler (1944), which consistently forms uniseptate conidia at 20°C, also forms many nonseptate ones at 28–32°C. Van Oorschot (1995) has noted that the identity of *A. arthrobotryoides* (Berl.) Lindau in its original sense cannot be determined from the protologue and cannot be resolved because of the absence of type material.

Arthrobotrys latispora is characterized by its broad, ovoid or slightly oval, partly nonseptate conidia, which are borne on nodes. Based on conidial shape, *A. latispora* most closely resembles *A. arthrobotryoides* sensu Drechsler and *A. amerospora*. The conidia of all three species are ovoid but differ in conidial size and septation. Differences between *A. latispora* and the other six known species that form non-septate conidia are summarized in Table 1.

Three additional collections also made on 20 September 2008 in the same region were associated with the same teleomorph but produced a very different anamorph referred to the genus *Anguillospora*. The question arises as to whether *A. latispora* is a contaminant, a conclusion supported by the fact that a similar, closely related teleomorph, *O. luteorubella* (Nyl.) P. Karst., also produced a similar *Anguillospora* anamorph (Pfister 1997).

Despite the nearly identical conidial morphology of *A. latispora* and *A. botryospora*, our molecular phylogenetic analysis pointed out a clear difference

between these two species: the ITS1+5.8S+ITS2 rDNA sequence similarity between *A. latispora* and a non-type isolate of *A. botryospora* is 93.7%. Thus *A. latispora* and *A. botryospora* can be considered two distinguishable species.

The phylogenetic analysis also showed that *Dactylellina* species were basal to both *Drechslerella* and *Arthrobotrys*. These results support the hypothesis of Li et al. (2005) that the trapping devices of *Drechslerella* species (with constricting rings) and *Arthrobotrys* species (with adhesive networks) developed independently from adhesive knobs (ancestral types of trapping devices specific to *Dactylellina*). Moreover, the analysis indicates that *Arthrobotrys* species may have evolved from *Dac. haptospora* relatives, whereas *Drechslerella* species evolved from *Dac. phymatopaga* relatives. More studies are needed to investigate the evolutionary process of these predacious fungi.

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